82. A method according to claim 80, wherein said genetic variation is in a gene indicative of a genetic disease.--

REMARKS

Prior to submission of the amendments presented above, claims 1-20 were pending in the application. Claims 1-20 are canceled herein without prejudice or disclaimer, and claims 21-82, including independent claims 21, 44, and 66 have been added to recite more clearly that which applicant regards as his invention.

The cancellation of claims is made herein without prejudice or disclaimer of the subject matter recited therein, and applicant expressly reserves all rights to such subject matter. No new matter is introduced by these amendments. Claims 21-82, including independent claims 21, 44, and 66, thus are pending for examination, which are respectfully requested in view of the foregoing amendments and following remarks.

Support for Amendments

The new claims point out more clearly and distinctly that which applicant claims as his invention. Each new claim is supported by the specification as described below.

"Channels"

The term "channels" finds support throughout the specification, as shown by the following exemplary statements.

Page 1, line 18 *et seq.*: ("[t]he present invention provides a novel flow-through genosensor, in which nucleic acid recognition elements are immobilized within densely packed pores or channels").

Example 1, page 14, lines 10-14: (nanochannel glass ("NCG") wafers contain "a regular geometric array of parallel holes or channels as small as 33 nm in diameter or as large as several micrometers in diameter [that]. . . can possess packing densities in excess of 3 X 10^7 channels per square centimeter"). Page 15, lines 3-5 and 8-9: (two typical channel diameters are 450 and 300 nm, respectively.)

Example 3, page 18, lines 19 - 27: (porous silicon can be fabricated with "dense oriented arrays of pores" having a wide range of diameters or that are rectangular.)

"Binding reagents immobilized on the walls of . . . channels"

The term "binding reagents immobilized on the walls of. . . channels" finds support throughout the specification, as shown by the following exemplary statements.

Subparagraph "(m)" at page 9, line 15 *et seq.*: ("A microfabricated device, . . . comprising:(1) a substrate . . . (2) an array of discrete and isolated regions arranged across a surface of said substrate and extending there through to a second surface of said substrate, thereby forming pores in said substrate; (3) substantially homogeneous samples of a predetermined set of biomolecules . . . being fixed in one or more of said regions, such that one or more of said molecules is capable of binding with a molecular species passing there through.")

Page 13, lines 7-9: (a "porous wafer containing 0.1-10 micron diameter channels comprising the bonding region for biomolecules fixed therein.")

Page 15, line 7 *et seq.* (describes "DNA binding capacity" and compares increased binding of probes to insides of channels with amounts that can be bound to a flat surface. 10^8 probes can be bound to a 50 micrometer square area of flat surface, whereas 10^{10} to 10^{11} probes can be bound within a 50 micrometer cube of a porous silicon wafer. Similarly, whereas "at least 10^7" longer, plasmid molecules can be attached per square mm of flat glass, 10^9-10^{10} plasmid molecules can be immobilized per square mm of cross section of a porous wafer.

Page 1, line 28 *et seq.*: ("vastly increased surface area . . . per cross sectional area" for immobilizing binding reagents.) – DNA not binding reagent

Page 16, line 4: ("The DNA sample is flowed into the porous regions of the chip and incubated" indicating that hybridization of target to probe occurs in the channels of the chip, where the probes are immobilized.)

Page 20, line 5-7: ("The epoxysilane-amine linkage procedure described in EXAMPLE 4 is then carried out to covalently attach amine-containing biopolymer species to the walls of the pores.")

Page 32, lines 19-20: ("The pores of the wafer are activated to bind amine-derivatized polynucleotides by reaction with epoxysilane, as described in EXAMPLE 4.")

"Binding reagent"

The term "binding reagent" finds support throughout the specification, as shown by the following exemplary statements.

Title of the application: "* * Apparatus for Discrete Detection of Binding Reactions.")

Page 2, lines 12-13: ("the present invention provides . . an improved apparatus and method - for simultaneous conduct of . . . binding reactions on a substrate." Binding reactions inherently require binding reagents, and a wide variety of reagents used for binding reactions are disclosed in the specification, for example at page 4, line 25 through page 5 line 3 and at page 12, line 25 through page 13 line 2).

OK

Dimensions and densities of wafers and channels

The dimensions and areas recited in the claims are supported throughout the specification, which describes the dimensions of wafers and channels, channel spacing, and the arrangement of channels in substrates. Cross sectional surface areas are dictated by diameters, and can be calculated from the diameters by simple geometrical formulae. Similarly, surface areas inside channels may be calculated using the known channel diameters and wafer thicknesses. The percentage of substrate surface area occupied by channels can be calculated using the dimensions of the channel and the distance between the channels.

Page 14, line 10: (The channels may have diameters as small as 33 nm up to "several micrometers.")

Page 18, line 3: (Channels in porous silicon wafers can have diameters "from 2 nm to several micrometers.")

Page 19, line 15: (Channels typically have diameters of 0.2 micrometers.)

Page 13, lines 7-8: (Porous wafers "containing 0.1 to 10 micrometer diameter channels." Cross-sectional areas of channels can be calculated directly from diameters.)

Page 14, lines 28-30: (wafers can be 0.1 to 10 micrometers thick.)

Page 15, lines 11: (450 nm diameters of channels, 750 nm spacing between channels - provides cross-sectional area that is about 28% channels and about 72% solid substrate. Ratios of (channel surface area):(cross sectional area of the substrate) can be calculated from channel diameters, substrate thickness, and the diameter of the group of channels.)

Page 25, lines 8-20: (This section discusses cross sectional areas of groups of channels in which DNAs are immobilized, and states that about 10^8 oligonucleotide molecules can be immobilized in a 50μm X 50μm area of flat surface, but that 10^{10} to 10^{11} molecules can be immobilized in 50 μm cube of porous silicon. Similarly, at least 10^7 molecules of plasmid pBR322 can be immobilized per mm^2 of flat glass surface, 10^9 to 10^{10} molecules of pBR322 can be immobilized per mm^2 of cross sectional area of nanoporous substrates.

Types of binding reactions

See, *inter alia*, page 4, line 25 through page 5 line 3; page 12, line 25 through page 13 line 2.

Groups of channels

Claim recitations relating to groups of channels with immobilized binding reagents are supported generally throughout the specification. See, for example, page 15, line 6-11. In addition, the specification illustrates groups of channels defined by immobilization of binding reagents in discrete and isolated regions, such as those defined by the wells in the manifold depicted in Figure 1.

Detecting expression of at least one gene

Methods of detecting gene expression are described at page 12, lines 25-27, and in Example 11. Methods of detecting expression between normal and mutated states of a cell or tissue are described in Example 10 and Figure 5 of the specification. Methods for detecting differences in gene expression in cells exposed to a drug or other chemical compound are described at page 34, lines 2-5, and in Figure 6.

Methods of detecting sequence variation

Methods of detecting sequence variation are described in Example 10 and Figure 5 of the specification. Methods of detecting variations that are indicative of genetic disease are described at page 11, lines 12-24 of the specification.

Discrete channels

Claim recitations to "discrete channels" are generally supported throughout the specification. See also Figures 1-4 and Example 1, and the discussion set forth below.

The Term "discrete and isolated"

The specification of the instant application applies the term "discrete and isolated" to several different types of "regions" of the claimed devices. For example, one such "region" is the pore or channel that extends through a substrate. Another example is the region defined by the group of channels where a given binding reagent is immobilized. This second type of "region" is defined, therefore, by the presence of the binding reagent. Methods of demarcating a region in this fashion may use, for example, a spotter, such as an ink-jet dispenser, that determines where each binding reagent is immobilized on the substrate. Similarly, a manifold can determine where binding reagents contact and are immobilized in a substrate. The present application states that a suitable manifold may, for example, be bonded to a substrate or may be integral.

In addition, page 15, lines 7-11, of the specification states that "separated clusters of channels" may be "formed during the [substrate] fabrication process." It will be understood that such separated clusters are structural features that also are "discrete and isolated" regions. It also will be understood that a "discrete and isolated" region can be defined by the sample wells in a manifold that determines where samples contact a substrate.

In sum, "discrete and isolated" regions may be formed in several ways within the context of the claimed invention. It should be noted that substrates of the claimed invention have discrete channels extending through the substrate. These discrete channels are only one type of "discrete and isolated region" set forth in the disclosure. The claims also recite use of first and second binding reagents that are immobilized in first and second groups of the discrete channels. A group of channels defined by the presence of a binding reagent is a second type of discrete and isolated region. Such channel groups can be defined, for example, by "separated clusters" of channels that are fabricated in the substrate. Other ways to define such groups of channels include use of a manifold for distributing binding reagents or samples to discrete and isolated regions of the substrate. The skilled artisan readily will appreciate other ways in which such regions can be formed.

CONCLUSION

In view of the foregoing, it is respectfully urged that the present claims are in condition for allowance. An early notice to this effect is earnestly solicited. Should there be any questions regarding this application, the Examiner is invited to contact the undersigned at the number shown below.

If any additional extension(s) of time are required for the filing of this paper, applicant expressly petitions for such extension(s) and authorize the Commissioner to charge any deficiency to Deposit Account 19-0741.

Respectfully submitted,

8/27/98
Date

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The Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 19-0741